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Ecology of the gut microbiota of *Tracheoniscus rathkei* (Crustacea, Isopoda)

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1. Introduction

A potentially important but little studied microbial habitat in natural ecosystems is the intestinal tract of invertebrates. The presence of freshly macerated organic matter in a moist gut that contains depolymerizing enzymes would seem to be a favorable environment for microbial development, and in this regard not unlike the guts of higher animals, which are widely known to contain an abundance of microbiota. In other studies, we have shown that woodlice and soil microorganisms together were more efficient in degrading ^{14}C-labeled yeast (REYES and TIEDJE 1973) and ^{14}C-labeled plant materials (REYES and TIEDJE 1976) than each separately. Since one possible site of animal-microbial interaction in organic matter decay is the intestinal tract, this study was conducted to determine the density, composition, population dynamics, and digestibility of the gut microbiota in an effort to better understand the role of these microorganisms in carbon metabolism.

2. Materials and methods

2.1. Estimation of microbial population in the animal gut

Tracheoniscus rathkei BRANDT, a terrestrial isopod commonly found in northern deciduous forests, was collected from campus woodlots and reared in the laboratory on a diet of decomposing wood found at the collection site. Other rearing details have been previously described (REYES and TIEDJE 1973).

The gut of the animal was aseptically extracted by holding the head of the animal with a sterile fine tipped forceps and pulling the last abdominal segment with another forceps; this operation was done in sterile plastic Petri dishes in a laminar air flow hood. The removed gut (not including the hepatopancreas) was macerated in 1 ml of sterile distilled water containing five glass beads (3 mm diameter) and vigorously shaken with a vortex mixer for 1 min. Leaf or wood chip samples were homogenized in the same manner but for 3–5 min depending on the size. The aqueous suspension was serially diluted up to 10^{-5} times in sterile distilled water prior to pour plating in nutrient agar and/or in leaf extract agar. Duplicate plates of each dilution were made. Leaf extract agar was prepared using 1.5% agar, 0.1% glucose and 1% stock extract solution obtained from 20 g (dry weight) of freshly collected leaf litter autoclaved with 500 ml of distilled water for 15 min at 15 psi. Nutrient agar gave the highest colony counts among the several media tested and thus was used routinely for most experiments.

For samples that needed expression of results in microbial count per unit weight of sample, 0.1 ml of the macerated sample was pipetted into a preweighed 1 cm-diameter aluminum foil disc that had been heated to 110 °C to constant weight and cooled in a desiccating jar. The sample was dried at 60 °C for 24 hr and weighed on a microelectrobalance (Cahn Instrument Co., Paramount, Cal.). The weight of glass from attrition during maceration was corrected by subtracting the dry weight of 0.1 ml from test tubes that contained water only. In the case of populations expressed per weight of gut contents, an additional correction was made by subtracting the mean weight of macerated gut wall obtained from gut samples in which the contents were previously dislodged by gentle shaking.

2.2. Effect of laboratory rearing and starvation on the gut microflora

Ten animals being reared in the laboratory on natural substrates were sacrificed at each sampling period and the platable microbial population determined in the manner described above with nutrient agar and leaf extract agar as plating media. The sampling periods occurred after the animals had been in captivity for 1, 10, 41 and 50 days.

The effect of starvation on the gut population was studied using fifteen animals that were allowed to feed for 24 hr on decayed woody materials collected from the forest. Subsequently, the animals were transferred to sterile Petri dishes without food and the dishes incubated inside a plastic box lined with moist filter paper. Five animals were sacrificed after 12, 36 and 72 hr of incubation and the microbial population of the gut contents determined.

2.3. Aerobic and anaerobic growth of the gut microflora

The fraction of gut flora that was able to grow anaerobically when initially grown aerobically or was able to grow aerobically when first grown anaerobically was determined by the replica plating technique. The samples that were initially incubated anaerobically were prepared under strict anaerobic conditions using the Hungate anaerobic technique (HUNGATE 1969). Freshly autoclaved distilled water to be used in serial dilutions was cooled, 0.05% cysteine was added as a reductant and the tubes were sealed with rubber stoppers. The test tubes were ascertained to be anaerobic by running a parallel control tube which contained 0.004% resazurin as a redox indicator (−51 mv). The samples were then surface plated on a prehardened Brewer anaerobic agar (Difco Laboratories, Detroit, Mich.) which had equilibrated for 48 hr inside an anaerobic plastic glove box (Coy Manufacturing Co., Ann Arbor, Mich.) described by ARANKI et al (1969). Anaerobiosis inside the chamber was confirmed by the reduced color of the resazurin indicator present in Brewer anaerobic agar. After 3 days, plates with approximately 30 colonies were replica plated onto another Brewer anaerobic agar plate and a nutrient agar plate inside the anaerobic box; the plates were incubated aerobically at 25 °C. A parallel experiment was conducted but samples were first incubated aerobically on nutrient agar plates. Replica plating was done onto Brewer anaerobic agar and nutrient agar plates as before but the plates were incubated anaerobically inside the glove box.

2.4. Microbial growth in the gut and faeces

The platable microbial count of leaf discs randomly cut with a No. 6 cork borer from leaf litter samples was determined. The gut and fresh faeces were also assayed for microbial counts using five randomly selected animals. Autoclaved (25 min at 15 psi) and non-autoclaved leaf discs were fed to another five animals and the microbial count of the uneaten food, the gut contents and faeces were determined after three or more faecal pellets had been excreted by the animals.

2.5. Effect of antibiotics on the gut microflora

A variety of antibiotics were examined for their ability to reduce the gut population of woodlice. Using a 10^{-2} dilution from five guts extracted as previously described, 0.4 ml of the suspension was spread on the surface of predried (2 days) nutrient agar plates. Separate antibiotic sensitivity discs(BBL, Cockeyville, Maryland) containing tetracycline (30 μg), chlortetracycline (30 μg), oxytetracycline (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), lincomycin (2 μg), dihydrostreptomycin (10 μg), penicillin G (10 units), polymixin B (300 units), and bacitracin (10 units) were pressed lightly on the surface of the agar. Discs were variously positioned on different plates to detect any synergistic combinations. The plates were equilibrated in a refrigerator at 5 °C for 2.5 hr, then incubated at 28 °C for 24 hr and finally evaluated for growth inhibition.

For feeding studies, in order to minimize chances of creating antibiotic toxicity to the animal, only two of the most effective antibiotics were chosen for use. To test the effectiveness of this combination, five randomly selected animals were dissected and the gut contents diluted and the microflora plated on nutrient agar. A second group of five animals was fed with 10 μl of a mixture of 500 ppm each of tetracycline and chlorotetracycline absorbed in 6 mm³ propylene oxide sterilized wood chips that had been dried at 60 °C overnight. The gut population was determined 2 days after the initial feeding.

2.6. Fate of microorganisms in the gut

Two bacteria that produced colonies which frequently occurred on plates and were easily distinguished were isolated and later fed to the animal to determine the fate of these organisms in the gut. One of the isolates was a Gram negative rod (1.4×0.9 μm), motile, aerobic, and non-pigmented. It did not produce acid or gas, reduced nitrate, did not produce indole or liquefy gelatin. The second isolate was a Gram negative rod ($2.0 \times 0{,}8$ μm), non-motile, aerobic, and produced a non-soluble yellow pigment. It did not produce acid or gas, reduced nitrate, did not produce indole and liquefied

gelatin. Based on these characteristics, the isolates were identified as species of *Pseudomonas* and *Flavobacterium*, respectively (SKERMAN 1967; BUCHANAN and GIBBONS 1974).

The isolates were grown in 100 ml of medium containing yeast extract, 1 mg; glucose, 50 mg; K_2HPO_4, 160 mg; KH_2PO_4, 40 mg; NH_4NO_3, 50 mg; $MgSO_4 \cdot 7\ H_2O$, 20 mg; $CaCl_2 \cdot 2\ H_2O$, 2.5 mg; and $FeCl_3 \cdot 6\ H_2O$, 2.5 mg. In the case of *Flavobacterium*, the medium contained 250 μC of ^{14}C-uniformly labeled glucose.

The cells were harvested by centrifugation and washed three times with cold sterile distilled water. The cells were resuspended to a final concentration of 158×10^4 cells of *Flavobacterium* per μl (0.002 μC/μl) and 232×10^4 cells of *Pseudomonas* per μl.

Dry propylene oxide sterilized wood chips (6 mm^3), on which 5 μl each of *Pseudomonas* and labeled *Flavobacterium* (0.01 μC) suspensions were absorbed, were individually fed to five animals that had previously received an antibiotic mixture of 5 μg each of chlortetracycline and tetracycline. The antibiotics were carried in 10 μl of sterile distilled water absorbed in wood chips. The respired, faecal, and assimilated labels were measured after 1 week by the method used in a previous study (REYES and TIEDJE 1973). The unconsumed label in the chip and the microbial counts in the gut, faeces and wood chip were determined. Wood chips with 10 μl of sterile water added and fed to animals served as the control.

3. Results

3.1. The gut flora

The gut population that grew on nutrient agar plates remained rather constant during a 50-day rearing period in the laboratory when fed natural food (Table 1). The constancy of the population is also indicated by the fact that a similar percentage of the nutrient agar population grew on leaf extract agar throughout the 50-day period.

Table 1. Effect of laboratory rearing on the microbial composition of the woodlouse gut

Time in captivity (days)	Plate count/gut $\times 10^{-3}$*		% on leaf extract
	Nutrient agar	Leaf extract	
1	112.7 ± 35	20.6 ± 2	18.3
12	260.4 ± 36	65.2 ± 15	25.1
41	128.6 ± 82	31.1 ± 14	24.2
50	221.7 ± 50	48.9 ± 20	22.1
Average	180.9 ± 72	41.5 ± 17	23.0

* Mean of ten animals ± standard error.

The portion of the gut population that grew aerobically and, following replica plating grew anaerobically is shown in Table 2. Though there is considerable variation in the density of the aerobic population, the facultatively anaerobic population exhibited a rather constant density and in some cases was dominant. This suggests that facultative anaerobes may be the more stable gut microbiota. No obligate anaerobes were apparent since all organisms that grew in the initial anaerobic incubation also grew aerobically.

Table 2. Physiological type of microorganisms found in the gut of woodlice

Animal	Plate count/g dry wt of gut contents $\times 10^{-8}$		
	Aerobic	Anaerobic*	% Anaerobic
A	75.7	6.2	8.2
B	58.1	16.8	28.9
C	17.7	14.1	79.8
D	8.3	7.3	87.5
Average	40.0	11.1	51.1

* Facultative.

As shown in Table 3, the population decreased 60-fold during a 72 hr starvation period. Since the data are expressed as population/g dry wt of gut contents, it indicates that the viable population relative to gut residue has dropped sharply probably due to lysis and assimilation.

Table 3. Effect of starvation on the microbial population of woodlouse gut

Time after last feeding (hr)	Plate count/g dry wt of gut contents* $\times 10^{-8}$
12	41.4 ± 23.1
36	4.2 ± 2.8
72	0.7 ± 0.4

* Mean of five animals $\pm$ standard error.

3.2. Microbial changes during passage of food through the gut

Table 4 shows the platable count of microorganisms per gram of material. The increase in population density from leaf to gut to faeces for collected animals indicates microbial growth. In addition the larger population increase after feeding leaf materials also suggests microbial growth during food passage through the animal. When the animals were fed autoclaved leaves, the population between leaf and faeces increased 600-fold. Fungi, which had reinfested autoclaved leaves, were also found in the gut but had disappeared from the faeces. Significantly, the major bacterial colony types were similar in the gut and faeces but were absent from the leaf material.

Table 4. Microbial density of leaf, gut and faeces during passage through woodlice

Treatment	Plate count/g dry wt $\times 10^{-8}$*		
	Leaf	Gut contents	Faeces
As collected	2.8 ± 1.0	6.3 ± 2.4	103.8 ± 43.2
After feeding			
Natural leaves	2.4 ± 1.4	16.2 ± 4.6	401.1 ± 85.3
Autoclaved leaves	1.1 ± 0.5 (2.3 ± 0.9)**	4.8 ± 2.1 (3.0 ± 1.2)	671.9 ± 88.7 (0)

* Mean of five samples $\pm$ standard error.
** Values in parenthesis are fungal colony counts from the same plates as the bacterial counts

3.3. Fate of microorganisms in the gut

Of the several antibiotics tested in this experiment, the broad spectrum antibiotics, the tetracyclines, produced the greatest inhibition of growth of the platable gut flora (Table 5). When the combination of tetracycline and chlortetracycline was fed to woodlice, the gut population dropped from an unusually high 5.8×10^7 platable organisms per gut in untreated animals to 3.7×10^4 organisms per gut in the treated animals, or approximately a 1000-fold reduction in population density. This reduction was deemed sufficient to attain the desired low level of population for experiments requiring reduced microbial densities in the gut.

When a dominant gut and faecal bacterium, *Flavobacterium*, was reintroduced in wood chip material as uniformly-^{14}C-labeled viable cells, the majority of the cells were metabolized as indicated by the high percentage of respired and assimilated label (Table 6). The respired $^{14}CO_2$ was primarily due to animal respiration since a large portion of the label was also assimilated though respiration by the *Flavobacterium* and by other gut flora may also have contributed to the total $^{14}CO_2$ collected.

Table 5. Effect of antibiotic on growth of the platable population from the woodlouse gut as determined by sensitivity discs

Antibiotic	Growth of gut flora*
Tetracycline	0
Chlortetracycline	0
Oxytetracycline	0
Chloramphenicol	+1
Erythromycin	+2
Lincomycin	+3
Dihydrostreptomycin	+1
Penicillin G	+3
Polymixin B	+2
Bacitracin	+3

* Growth response scale: 0 = no growth to +3 = no inhibition.

Table 6. Fate of ^{14}C-labeled *Flavobacterium* fed to woodlice for one week

Fate	% of ingested label	Plate count/gut $\times 10^{-4}$*	
		Expected	Actual
Respired	40.3	—	—
Assimilated	32.9	—	—
In gut	5.0	13	9 ± 6
In faeces	21.8	55	51 ± 6
Wood chip**	—	385	117 ± 11

* Mean of five animals ± standard error.

** 48.7% of the original label remained in the wood chip after the experiment (non-ingested label)

Table 7. Recovery of isolates and natural population fed to woodlice for one week

Source	Not-inoculated	Inoculated with isolates*		
	Total population	Total population	Pseudomonas	Flavobacterium
Plate count/gut $\times 10^{-4}$**				
Gut	27 ± 11	61 ± 15	34 ± 12	9 ± 6
Faeces	626 ± 134	279 ± 43	157 ± 42	51 ± 6
Wood chip	696 ± 126	643 ± 70	515 ± 34	117 ± 11

* Inoculum contained 1162×10^4 Pseudomonas cells and 790×10^4 Flavobacterium cells absorbed in wood chips.

** Mean of five animals ± standard error.

Based on ingested label and recovered label in the uneaten food, the expected population in the gut and faeces was 13 and 55×10^4 organisms/gut respectively. These values were very close to the actual count (Table 6). In contrast the population of the wood chip declined 3-fold during the week incubation period, indicating the chip was a less favorable environment. The simplest explanation is that the remaining label in the gut is contained in viable cells and that other viable and nonviable cells have been digested and their label contents assimilated or excreted. Table 7 provides the recovery values for both isolates and the total population compared to the natural population present in the control. In the antibiotic treated (inoculated) animals, it is apparent that both inoculated species and naturally reinfesting species followed the same pattern. The final total population values for the inoculated animals compares favorably with the values for the non-inoculated animals. Particularly for the inoculated animals, the trend of higher counts in the food and lower counts in the faeces suggests digestion of bacteria in the gut.

4. Discussion

Plant materials are extensively colonized by microorganisms during both living and decay stages, however, the successful species in these stages would not be expected to be the successful species in the gut of a soil animal because of the difference in the two environments. The possible fates of the microbial species entering the gut are growth, lysis and elimination. Those that established themselves in the gut could maintain a quasi-steady-state population depending on their rate of multiplication, availability of substrate, rate of washout and susceptibility to digestive enzymes. Since a more or less constant and distinct population was present in the gut during a 50-day rearing period in the laboratory, such a steady-state population of resident flora is suggested. Considering the 99 % reduction in population during starvation, favorable growth conditions appear necessary if the gut flora is to maintain itself against digestion and elimination. Other evidence that microorganisms may be multiplying in the gut is the increase in density of bacteria during the passage of the food from leaf to gut to faeces, both in freshly collected and laboratory fed animals. In addition, the difference of major colony types in the food compared with the gut and faeces suggests that there was digestion and growth of different flora at the same time.

Digestion of organisms in the gut is indicated by the reduction in population density during starvation. Mechanisms triggered by starvation such as increased digestive enzyme activity or autolysis could be responsible for greater microflora digestion. Woodlice are known to rest intermittently for 24—48 hr periods after feeding (Cole 1946) to allow more time for digestion of food. Singh (1971) observed that there was less bacteria and an absence of protozoa in collembola after starvation when compared to feeding animals. Microflora digestion was also implicated by Fredeen (1964) who found that blackfly larvae developed to adults only when fed with washed suspensions of bacteria.

The two bacteria, *Pseudomonas* and *Flavobacterium*, that were isolated and reintroduced into the animals are representatives of common genera found in plants and organic residues at various stages of decomposition (Holm and Jensen 1972; Last and Warren 1972; Graeves 1973). These particular species appear to be different from the dominant litter flora. The respiration of ingested label from the *Flavobacterium* again gives evidence that a gut resident could be utilized by the animal. The *Pseudomonas* probably had the same fate as the *Flavobacterium* according to their relatively similar pattern of distribution in the gut, faeces and uneaten food. The difference between the expected number of recoverable *Flavobacterium* cells and the actual count observed suggests that digested dead cells were the origin of the respired and assimilated label in the animal. The ease of digestion of normally successful gut microflora appears to be due to this experiment being carried out under conditions of starvation, i.e., the bacteria fed were absorbed on highly weathered wood chips.

Bayne (1973) made a similar study on land snail *Helix pomatia* (L.) by injecting ^{14}C-labeled bacteria *Serratia marcescens* and found that the bacteria declined rapidly inside the snail, especially after starvation. He concluded that the bacterial cells were either phagocytosed or degraded and the label incorporated into the snail tissue. Soil animals, particularly woodlice, do not have such an elaborate digestive system but do have certain digestive enzymes such as carbohydrases (Rajulu 1970), cellulase (Hartenstein 1964, Kühnelt 1961, Vonk 1960), chitinase (Rajulu 1970, Kühnelt 1961, Vonk 1960) and proteinases (Bewley and Devilles 1968). In addition, ultrastructural studies of hepatopancreas of woodlice revealed the secreting nature of the cells lining the interior wall of the organ (Donadey and Besse 1972, Clifford and Witkus 1971).

The importance of facultative anaerobes in the gut is consistent with expected low oxygen conditions due to restricted gas transport and active respiration. The absence of obligate anaerobes suggests that the gut is not constantly anaerobic which is also consistent with the simple tube-like nature of the gut of woodlice and much different from the more differentiated digestive systems of termites (Breznak 1975) and crane fly larvae (Klug and Kotarski 1974) which have more densely populated hind guts containing obligate anaerobes. Boll weevils which have a gut anatomy more similar to woodlice were also found to have a popu-

lation density and microbial types similar to woodlice (BRACKE and MARKOVETZ 1974). In a study of lepidopteran larvae, KINGSLEY (1972) found that the gut inhabitants were facultative anaerobes and that the number of aerobes and anaerobes fluctuated relative to each other. Although BURTON et al. (1973) did not account for anaerobes due to lack of material, they found that the aerobic microbial population in the midgut of *Simulium damnosum* larvae ranged from 8.9×10^4 — 400×10^4.

The hypothesis that the intestinal tract of a soil animal is a favorable environment for microbial growth is supported by the present study. However, it is also apparent that the animal is capable of digesting the microflora and assimilating the contents, particularly during starvation. Thus, a dynamic turnover of microbial biomass occurs. In other studies we showed that the relative contribution of gut microbial activities to organic matter digestion by the animal was minimal (REYES and TIEDJE 1976); however, the utilization of microbial biomass may be important particularly during periods of animal starvation.

5. Summary · Zusammenfassung

Freshly collected isopods kept in the laboratory for 50 days on a natural wood diet maintained a rather constant population of about 18×10^4 platable microorganisms per gut. Aerobes and facultative anaerobes were prevalent, but no obligate anaerobes were recovered. Starvation or treatment with antibiotics drastically reduced this population, suggesting that microorganisms must be growing to maintain their population against digestion and elimination. Microbial growth during passage of food through the gut is indicated by an increase in bacterial density from leaf to gut to faeces, and by differences in dominant colony types in the gut and faeces compared with the food. Two dominant members of the gut community were Pseudomonas and Flavobacterium species. ^{14}C-labeled Flavobacterium cells fed to woodlice were extensively digested under nutrient-limiting conditions with the contents being assimilated and respired by the animal. Resident gut microorganisms appeared to grow in the woodlouse gut, but they also appeared to be digested, particularly under starvation conditions.

Frisch gesammelte Isopoden, die im Labor 50 Tage lang bei natürlicher Holz-Diät gehalten wurden, enthielten etwa 18×10^4 Mikroorganismen pro Darmtrakt. Aerobe und fakultativ Anaerobe waren weit verbreitet, aber keine obligat aeroben Organismen. Aushungern der Asseln oder Behandlung mit Antibiotika verringerte die Mikroben-Population drastisch; dies deutet an, daß die Organismen nur durch Vermehrung den Prozessen der Verdauung und Ausscheidung entgegenwirken können. Eine Zunahme der Bakterien-Dichte, von Blattnahrung zu Darm zu Kot, deutet auf Wachstum der Mikroben während der Darmpassage von Nahrung hin, in gleichem Maß wie die Verschiedenheit der dominanten Kolonietypen in Darm und Kot, verglichen mit der frischen Nahrung. Zwei dominante Darmbewohner waren Pseudomonas- und Flavobacterium-Arten. Mit ^{14}C markierte Flavobacterium-Zellen, bei beschränkter Ernährung an Isopoden verfüttert, wurden von den Tieren assimiliert. So scheinen Mikroorganismen im Isopodendarm einerseits zu wachsen, andererseits aber werden sie auch verdaut, besonders wenn sich die Tiere im Hungerzustand befinden.

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7. Literature cited

ARANKI, A., S. A. SYED, E. B. KENNEY and R. FRETER, 1969. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. Appl. Microbiol. **17**, 568—576.

BAYNE, C. J., 1973. Molluscan internal defense mechanism: The fate of ^{14}C-labelled bacteria in the land snail *Helix pomatia* (L.). J. Comp. Physiol. **86**, 17—25.

BEWLEY, G. C., and E. J. DEVILLES, 1968. Isolation and characterization of the digestive proteinases in the earthworm *Lumbricus terrestris* LINNAEUS. Comp. Biochem. Physiol. **25**, 1061—1069.

BRACKE, J. W., and A. J. MARKOVETZ, 1974. Gut flora of the boll weevil. Abst. Ann. Meetings of Amer. Soc. Microbiol. p. 46.

BREZNAK, J. A., 1975 Symbiotic relationships between termites and their intestinal microbiota. In: Symbiosis. XXIX. Symposium of the Soc. for Exper. Biol. Cambridge Univ. Press. In press.

Buchanan, R. E., and N. E. Gibbons, 1974. Bergey's manual of determinative bacteriology, 8th ed., Williams and Wilkens Co., Baltimore. Md. pp. 1246.
Burton, G. J., I. V. Perkins and H. S. Sodhi, 1973. Aerobic bacteria in the midgut of *Simulium damnosum* larvae. Mosquito News. **33**, 115–117.
Clifford, B., and E. R. Witkus, 1971. The fine structure of the hepatopancreas of the woodlice, *Oniscus asellus*. J. Morphol. **135**, 335–349.
Cole, L. C., 1946. A study of the cryptozoa of an Illinois woodland. Ecol. Monogr. **16**, 50–86.
Donadey, C., and G. Besse, 1972. Histological, ultrastructural and experimental study of the digestive caeca of *Porcellio dilatus* and *Ligia oceanica* (Crustacea, Isopoda). Biol. Abst. **55**, 66885.
Fredeen, F. J. H., 1964. Bacteria as food for blackfly larvae (Diptera: Simuliidae) in laboratory cultures and in natural streams. Can. J. Zool. **42**, 527–548.
Greaves, H., 1973. Selected wood-inhabiting bacteria and their effect on strength properties and weights of *Eucalyptus regnans* F. Muell. and *Pinus radiata* D. Don sapwoods. Holzforschung **27**, 20–26.
Hartenstein, R., 1964. Feeding, digestion, glycogen, and the environmental conditions of the digestive system of *Oniscus asellus*. J. Insect Physiol. **10**, 611–621.
Holm, E., and V. Jensen, 1972. Aerobic chemoorganotrophic bacteria of a Danish beech forest. Oikos. **23**, 248–260.
Hungate, R. F., 1969. A role tube method for cultivation of strict anaerobes. In: J. P. Norris and D. W. Ribbons (eds.). Methods in Microbiology. Vol. 38. Academic Press, London. pp. 117–132.
Kingsley, V. V., 1972. Persistence of intestinal bacteria in the developmental stages of the monarch butterfly *(Danaus plexipus)*. J. Invert. Pathol. **20**, 51–58.
Klug, M. J., and S. F. Kotarski, 1974. Ecology of the intestinal microflora of larvae of an aquatic crane fly. Abst. Ann. Meetings. of Amer. Soc. Microbiol. p. 46.
Kühnelt, W., 1961. Soil Biology, Rodale Books, Emmaus, Pa. pp. 249–256.
Last, F. T., and R. C. Warren, 1972. Non-parasitic microbes colonizing green leaves. Their form and function. Endeavour **31**, 143–150.
Rajulu, G. J., 1970. Studies on the nature of the carbohydrases in a centipede *Scolopendra heros*, together with observations on hydrogen ion concentration of the alimentary tract. J. Anim. Morphol. Physiol. **17**, 56–64.
Reyes, V. G., and J. M. Tiedje, 1976. Metabolism of ^{14}C uniformly labeled organic material by woodlice (Isopoda: Oniscoidea) and soil microorganisms. Soil Biol. Biochem. **5**, 603–611.
Reyes, V. G., and J. M. Tiedje, 1975. Metabolism of ^{14}C-labeled plant materials by woodlice (*Tracheoniscus rathkei* Brandt) and soil microorganisms. Soil Biol. and Biochem. (in press).
Singh, S. B., 1971. A preliminary observation on the gut contents of *Neanura muscorum* (Templeton) (Collembola, Neanuridae). Entomol. Mo. Mag. **106**, 85–87.
Skerman, V. B. D., 1967. A guide to the identification of the genera of bacteria. Williams and Wilkens Co. Baltimore, Md. pp. 303.
Vonk, H. J., 1960. Digestion and metabolism. In: T. H. Waterman (ed.). The physiology of crustacea, Vol I, Academic Press, New York, p. 297.

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